

WHAT IS CLAIMED IS:

1. A method of monitoring cellular activity in a cellular specimen, comprising:  
applying a plurality of different excitable markers to the specimen;  
focusing light upon a region of the specimen from a laser microscope to excite the  
markers in the region and cause fluorescence to be radiated by the markers in the region;  
separating the fluorescence into wavelength bands using a tunable filter;  
detecting the fluorescence through an array of detectors, with each detector receiving  
one of the wavelength bands and generating a corresponding signal; and  
analyzing the detected fluorescence to qualitatively and quantitatively identify the  
contribution to the fluorescence from each of the plurality of different excitable markers.

2. The method of claim 1, wherein the step of analyzing includes quantitatively  
determining an intensity contribution to the fluorescence from each of the plurality of  
different excitable markers.

3. The method of claim 2, wherein the step of analyzing uses a linear unmixing  
operation.

4. The method of claim 3, wherein the linear unmixing operation includes the  
steps of:  
storing the detected fluorescence in a memory;  
comparing the stored fluorescence with a model fluorescence generated from a  
plurality of templates characteristic of a fluorescence spectrum of each of the plurality of  
different excitable markers;  
varying the weights of each of the plurality of templates until the model fluorescence  
closely matches the stored fluorescence; and  
determining quantitative information about the intensity contribution to the fluorescence  
from each of the plurality of different excitable markers based on the weights.

5. The method of claim 1, wherein the step of analyzing uses a principal  
component analysis of the fluorescence.

6.        The method of claim 1, wherein separating the fluorescence includes using one or more of either a grating or prism.

7.        The method of claim 1, wherein separating the fluorescence includes using a dichromatic mirror.

8.        The method of claim 1, wherein the tunable filter is a liquid crystal filter.

9.        The method of claim 1, wherein the tunable filter is an acousto-optical filter.

10.       The method of claim 1, wherein applying a plurality of excitable markers includes applying a plurality of fluorescent probes to the specimen.

11.       The method of claim 1, wherein detecting the fluorescence includes using a plurality of photomultiplier tubes.

12.       The method of claim 1, wherein detecting the fluorescence includes using a plurality of high gain photomultiplier tubes.

13.       The method of claim 1, wherein focusing light to the specimen comprises focusing light from a two-photon laser microscope.

14.       A system for monitoring cellular activity in a cellular specimen that contains a plurality of excitable markers, the system comprising:

        a laser microscope that is operative to excite the markers in a region of the specimen, wherein the markers in the region radiate fluorescence as a result;

        a tunable filter that is operative to process the fluorescence and to pass a portion of the fluorescence, wherein the portion of the fluorescence is within a wavelength band that depends on the setting of the filter;

        a plurality of detectors operative to receive the processed fluorescence and to convert the fluorescence into a corresponding plurality of signals; and

an analyzer that is operative to receive the signal and to qualitatively and  
quantitatively identify the contribution to the fluorescence from each of the plurality of  
5 different excitable markers.

15.     The system of claim 14, wherein the analyzer is operative to quantitatively  
determine an intensity contribution to the fluorescence from each of the plurality of different  
excitable markers.

10        16.     The system of claim 15, wherein the analyzer uses a linear unmixing  
operation.

17.     The system of claim 16, wherein the analyzer comprises:  
15        a memory operative to store the detected fluorescence;  
          a processor operative to generate a model fluorescence from a plurality of templates  
characteristic of a fluorescence spectrum of each of the plurality of different excitable  
markers, compare the model fluorescence with the stored fluorescence, vary the weights of  
each of the plurality of templates until the model fluorescence closely matches the stored  
20        fluorescence, and determine quantitative information about the intensity contribution to the  
fluorescence from each of the plurality of different excitable markers based on the weights.

18.     The method of claim 14, wherein the analyzer uses a principal component  
analysis.

25        19.     The system of claim 14, wherein the tunable filter comprises a liquid crystal  
tunable filter.

20.     The system of claim 14, wherein the tunable filter comprises an acousto-  
30        optical tunable filter.

21.     The system of claim 14, wherein the plurality of detectors comprise a plurality  
of photomultiplier tubes.

22.     The system of claim 14, wherein the plurality of detectors comprise a plurality  
of high-gain photomultiplier tubes.

23.     The system of claim 14, wherein the laser microscope comprises a multi-  
photon laser microscope.

24.     The system of claim 14, therein the system further comprises a collector that at  
least substantially envelops the specimen to receive fluorescence from the markers.

25.     The system of claim 24, wherein the collector comprises an integrating sphere.

26.     A laser scanning microscope for monitoring cellular activity in a cellular  
specimen that contains a plurality of excitable markers, the microscope comprising:

    a laser operative to excite the markers in a region of the specimen, wherein the  
markers in the region radiate fluorescence as a result;

    a tunable filter that is operative to process the fluorescence and to pass a portion of the  
fluorescence, wherein the portion of the fluorescence is within a wavelength band that  
depends on the setting of the filter;

    a plurality of detectors operative to receive the processed fluorescence and to convert  
the fluorescence into a corresponding plurality of signals; and

    an analyzer that is operative to receive the signal and to qualitatively and  
quantitatively identify the contribution to the fluorescence from each of the plurality of  
different excitable markers.

27.     The microscope of claim 26, wherein the analyzer is operative to  
quantitatively determine an intensity contribution to the fluorescence from each of the  
plurality of different excitable markers.

28.     The microscope of claim 26, wherein the analyzer uses a linear unmixing  
operation.